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GC-MS Metabolite Profiling and Chemometric Analysis of Robusta Green Beans (Bantjah Coffee)

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In this study, three types of Robusta green bean coffee developed in the Bantjah area were characterized. This study characterized three types of Robusta green coffee beans with potential for development in the Bantjah area. The three coffee beans were (A) Bantjah area and the two (B, C) selected from different altitudes of the West Sumatra area. The purpose of this study was to analyze metabolite profiling of Robusta green coffee green beans. The coffee beans were fermented using the natural method and then the extract was derivatized with N-Methyl-N-(trimethylsilyl) trifluoroacetamide before being analyzed by Gas chromatography-mass spectrometry (GCMS). The chromatogram obtained was then analyzed statistically using principle component analysis (PCA). GCMS analysis produced more than 60 chemical components in green beans of Robusta coffee. Principle component analysis determined the metabolite distribution of coffee samples as influenced by their geographical origin. Coffee originating from the highlands had different marker compounds than coffee grown in lower plains. A metabolomics approach provides a comprehensive explanation of this relationship.
Keywords: Agroecology, caffeine, highlands, aroma, metabolomics, PCA.

INTRODUCTION

The growing location and environmental factors such as temperature, humidity, light intensity, and soil type greatly influence the content of primary and secondary metabolites in coffee. Primary metabolites such as carbohydrates, proteins, and fats are formed as part of essential plant growth. They are strongly influenced by the availability of nutrients and environmental conditions that support photosynthesis and respiration. Meanwhile, secondary metabolites such as flavonoids, alkaloids, and essential oils are often produced in response to environmental stress conditions, such as drought, exposure to UV light, or interactions with microorganisms (Syukri *et al.,* 2024). For example, herbal plants grown in areas with low rainfall often show increased production of essential oils as an adaptation mechanism to dry environments (Yang *et al.,* 2018; Pant *et al.,* 2021). The metabolite profile in agricultural products is closely related to the formation of taste, because primary and secondary metabolite compounds contribute to the taste, aroma, and texture of a product. Primary metabolites, such as sugars, organic acids, and amino acids, play a role in forming basic tastes such as sweet, sour, and umami. For example, the sugar content in fruits such as mango or strawberry determines the level of sweetness, while citric and malic acids provide a distinctive acidity sensation. On the other hand, secondary metabolites, such as flavonoids, alkaloids, and essential oils, provide complexity of taste through bitter, spicy, or distinctive aroma sensations. For example, the content of alkaloids such as caffeine in coffee and theobromine in chocolate creates a bitter taste that is characteristic of the product, while essential oils in spices such as cloves and cinnamon provide a strong aroma and taste (Li *et al.,* 2020; Anggraini *et al.,* 2021; Yeshi *et al.,* 2022; Roy *et al.,* 2022; Elshafie *et al.,* 2023).

The growing location has a significant influence on the taste of coffee, as environmental factors such as altitude,

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temperature, rainfall, soil type, and light intensity affect the development of metabolite compounds in coffee beans. Coffee grown at high altitudes, such as above 1,200 meters above sea level, usually has a slower growth rate due to lower temperatures, allowing for a higher accumulation of sugars and organic acids, resulting in a more complex, sweeter, and more acidic flavor. In contrast, coffee from the lowlands tends to have a thicker body with lower acidity levels. Soil type also plays an important role, where mineral-rich volcanic soils, such as in the mountainous regions of Indonesia and Latin America, can increase the content of secondary metabolites that contribute to the distinctive aroma and flavor of coffee. In addition, rainfall patterns and sunlight exposure affect the balance between phenolic compounds and caffeine, which play a role in shaping the level of bitterness and other flavor characteristics. Therefore, each coffee-growing region has a unique flavor profile, such as the fruity acidity of Ethiopian coffee, the chocolate flavor of Brazilian coffee, or the spice nuances of Sumatran coffee, all of which are the result of a complex interaction between the growing location and the metabolism of the coffee plant (Ahmed *et al.*, 2021). Bantjah is a coffee product produced by the Sikayan Balumuik Community Forestry Group, located in the Bantjah area, Limau Manis village, Pauh district, Padang city, West Sumatra. Because Padang City is located in the lowlands, Bantjah coffee is a derivative of Robusta coffee products. Although Robusta coffee is known for its bitter taste, the taste of Bantjah coffee has succeeded in penetrating the national and international markets. Determining the quality of Robusta coffee is quite difficult because of the scarcity of Q graders. Therefore, the quality of Bantjah coffee is determined chemically. Generally, chemical analysis of a product is carried out by analyzing the macro components of the product such as carbohydrate, protein, fat, and water content. However, this analysis technique takes a long time and requires a fairly large number of samples.

Coffee has distinct flavor nuances depending on its geographic origin, and its chemical properties and ultimate quality are greatly influenced by its growing area. The amount of green beans produced at the start of the process (postharvest treatment) has a significant impact on how marker components emerge in coffee samples (De Bruyn *et al.*, 2016). The metabolic process under environmental stress affects the composition of green beans. The location of the growth area affects this stress situation. To identify the quality of Bantjah coffee in this study, analysis was carried out using gas chromatography-mass spectrometry.

MATERIALS AND METHODS

***Natural post-harvest processing procedure of coffee beans*:** The three types of coffee beans were (A) Bantjah area and the two (B, C) selected from West Sumatra area. The altitudes of A, B and C were 1-3 meters, 500-600 meters and 300-500 meters above sea level, respectively.

Coffee cherries from three different areas (A, B and C) were picked (20 Kg each). Coffee cherries were sorted using a tub filled with running water and the floating coffee cherries were separated. The red fruit was dried and then peeled, yet the coffee beans were with the skin. Bantjah coffee was processed with the natural method. The natural method is one of the methods used in the postharvest process of coffee to produce coffee flavor. During natural process, coffee cherries were cleaned, sorted and dried under the sun. Usually, the drying process is stopped once the moisture content reaches 12%. Once dried, the cherries underwent hulling to remove the parchment and silver skin, resulting in green coffee beans.

***Analysis of green bean components using GC-MS derivatization*:** The compounds in the sample were derivatized by silanization. The sample was weighed as much as 30 mg. Then added 1 mL of solvent mixture (methanol, chloroform, aquabides 2.5: 1: 1). After that, ribitol standard (0.2 mg / mL aquabides) was added as much as 60 µL. Then vortexed for 5 minutes and centrifuged for 10 minutes (13,000 g). Then 900 µL of supernatant was taken and 400 µL of aquabides was added. Then vortexed again for 5 minutes and centrifuged for 10 minutes. After that, the water phase (upper part) was taken into a test tube and evaporated using nitrogen gas. After that, methoxyamine hydrochloride dissolved in pyridine (20 mg / mL) was added as much as 100 µL. Then shaken for 90 minutes at a temperature of 37°C and a power of 150 rpm. After that add MSTFA as much as 50 µL and shaken again for 30 minutes. Ribitol is used as an internal standard. Samples (1 µL) were injected in split mode [12:1 (v/v)] at an injection temperature of 230 °C and analyzed. The GCMS of Shimadzu Japan was used for this study. The analysis was done with at least three replications (Rini *et al.*, 2021; Syukri *et al.*, 2023; Syukri *et al.*, 2024).

***Chemometric data processing*:** The data obtained from the results of green bean analysis (GCMS) were then extracted using GC solution software and then processed multivariate using Origin Pro software.

RESULTS

Figure 1 shows the chromatogram of Robusta green bean grown in the Bantjah (A), Solok (B) and South Solok (C) areas. Visually, the profile of the compounds detected from each sample is the same. The compounds detected were compounds resulting from silanization derivatization of the OH group (Caban and Stepnowski, 2018). Thus all polar compounds that have hydroxyl groups can be analyzed in this study. Table 1 shows the names of the most compounds detected in the chromatogram. From each chromatogram, the average number of compounds detected from the GCMS library was up to 60 compounds. These compounds were

tested for multivariate analysis to see if there are any compounds that differentiate each coffee sample used.

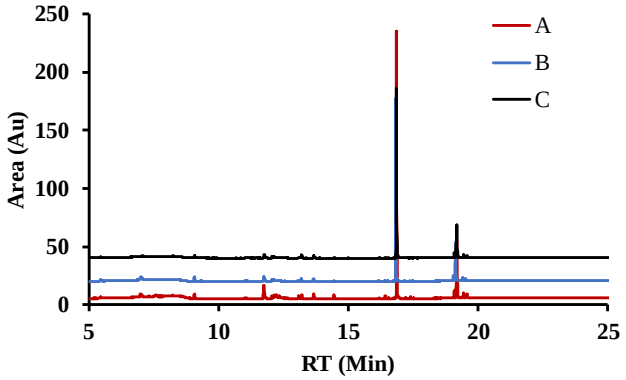

Figure 1. GCMS chromatoragm of Robusta coffee that is grown in three different places (A, B and C).

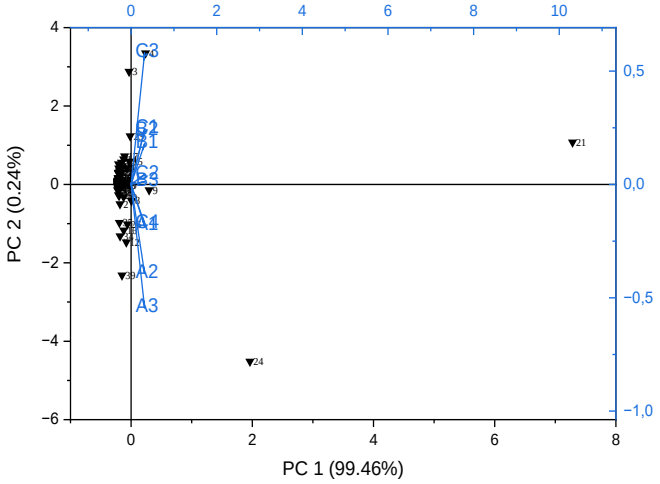

Figure 2. Metabolite distribution form green bean coffee from three different places (A, B and C).

Table 1. The most detected compound from all samples.

Sr.	Compounds Name
1	Pyridine
2	2,2,8,8-Tetramethyl-3,7-dioxa-4-aza-2,8-disilanon-4-ene
3	Methane Disulfonic Acid, di(TBDMS)-
4	Glycerol, 3TMS derivative
5	3H-1,2-Benzoxathiole, 5-nitro-, 2,2-dioxide
6	Benzoic acid, 5-(chlorosulfonyl)-2-methoxy-
7	N-(Trimethylsilyl)oxy-benzenesulfonamide
8	Malic acid, 3TMS derivative
9	Citric acid, 4TMS derivative
10	Quininic acid (5TMS)
11	Caffeine
12	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime
13	D-(+)-Talose, pentakis(trimethylsilyl) ether, methyloxime (anti)
14	Myo-Inositol, 6TMS
15	Palmitic Acid, TMS derivative
16	Scyllo-Inositol, 6TMS
17	Stearic acid, TMS derivative
18	D-(+)-Turanose, octakis(trimethylsilyl) ether
19	1-Monopalmitin, 2TMS derivative
20	Heptyl 1-thio-.beta.-D-glucopyranoside, 4TMS
21	Sucrose, 8TMS derivative
22	Glycerol monostearate, 2TMS derivative
23	cis-5-O-Feruloylquinic acid, 5TMS
24	Chlorogenic acid, 6TMS
25	4-O-Feruloylquinic acid, 5TMS
26	(1r,3R,4s,5S)-4-(((2E)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl)oxy)-1,3,5-trihydroxycyclohexanecarboxylic acid, 6TMS (isomer 1)
27	(6,8,9-Trimethyl-4-propyl-3-oxabicyclo[3.3.1]non-6-en-1-yl)methanol, TMS
28	Adonitol, 5TMS
29	Lactic Acid, 2TMS derivative
30	Oxalic acid, 2TMS
31	Trisiloxane, octamethyl-
32	Diethylene glycol, n-butyl ether, trimethylsilyl ether
33	2-Hexenedioic acid, bis(trimethylsilyl) ester, (E)-
34	D-(-)-Fructose, pentakis(trimethylsilyl) ether, methyloxime (syn)
35	D-Allose, pentakis(trimethylsilyl) ether, methyloxime (anti)
36	D-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxyme, (1Z)-
37	D-Glucitol, 6TMS
38	D-Psicofuranose, pentakis(trimethylsilyl) ether (isomer 2)
39	5-O-Feruloylquinic acid, 5TMS
40	((1R,4S,5R,8S,9R)-4-Isopropyl-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-en-1-yl)methanol, TMS
41	D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxyme, (1Z)-
42	4-Heptanone, 2-methyl-
43	4-Heptanone, 3-methyl-
44	2-Monostearin, 2TMS derivative
45	Linocinnamarin, 4TMS
46	2-Palmitoylglycerol, 2TMS derivative
47	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
48	2,3-Butanediol, O-(trimethylsilyl)-, monoacetate
49	Xylitol, 5TMS
50	2-(Phenylsulfanyl)acetohydrazide, 2TMS derivative

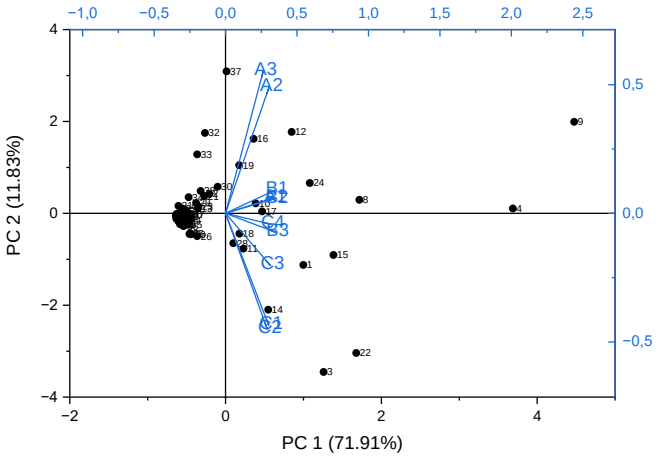

Figure 3. Extended Metabolite distribution form green bean coffee from three different places (A, B and C).

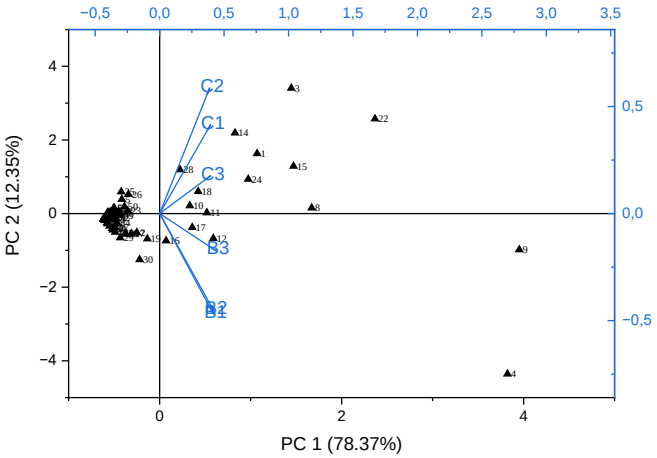

Figure 4. Extended Metabolite distribution form green bean coffee from two different places (B and C).

DISCUSSION

In theory, it has been explained that the growing location affects the composition of a compound in agricultural materials. The chemical composition of coffee beans is greatly influenced by the growing location, which includes factors such as altitude, soil type, temperature, rainfall, and sunlight intensity. One of the main components in coffee is caffeine, the levels of which can vary depending on the altitude of the growing place. Coffee grown at high altitudes, such as Arabica varieties, tends to have lower caffeine levels but is rich in volatile compounds that contribute to complex aromas and subtle flavors. In contrast, Robusta coffee grown at low altitudes has higher caffeine levels, giving it a more bitter taste and a stronger body. Additionally, the content of organic acids such as chlorogenic acid (the specific compound of roasted coffee), which acts as a natural antioxidant, is also influenced by environmental factors. Coffee grown in cooler climates tends to have higher levels of chlorogenic acid, which contributes to the characteristic acidity in the coffee's flavor profile. The essential oils and natural sugars in coffee can also change according to soil conditions and rainfall patterns, affecting the level of sweetness and richness of flavor (Olechno *et al.*, 2021). Therefore, growing location is an important factor in determining the chemical characteristics and final quality of coffee, making it a beverage with unique flavor complexities based on its geographic origin. The appearance of marker components in coffee samples is greatly influenced by the content of green beans produced at the beginning of the process. The content of green beans will be influenced by the metabolic process due to environmental stress. This stress condition is influenced by the location of the growing place.

Figure 2 shows the distribution of metabolites from the three coffee samples. It can be seen that the distribution of compounds cannot be separated well. The distribution profile seems to be affected by components no. 21 and 24 which dominate the clustering of compounds from the three types of samples. These dominant compounds are known as sucrose and chlorogenic acid. These compounds were also detected as the main peak on the GCMS chromatogram at the retention time of 17 and 19 minutes. Green coffee beans, or raw coffee beans in general, do contain various chemical compounds that play a role in determining the characteristics of the taste after roasting, including sucrose and chlorogenic acid. Sucrose is a natural sugar found in significant amounts in green coffee beans, contributing to the sweetness that develops during the roasting process through Maillard reactions and caramelization (Rini *et al.*, 2024). The higher the sucrose content in coffee beans, the more complex the aroma and sweet taste produced after brewing. Meanwhile, chlorogenic acid is a phenolic compound that dominates the acid content in green coffee beans. This compound is responsible for the acidity level of coffee and has antioxidant properties that are beneficial to health. However, during roasting, chlorogenic acids are degraded into bitter compounds such as caffeic acid and quinic acid, which contribute to the more bitter and complex flavor characteristics of coffee. The balance between sucrose and chlorogenic acids in green coffee beans is an important factor in determining the final flavor profile after roasting and brewing.

Since the distribution of metabolites cannot describe the correlation with the types of samples analyzed in this study, further analysis was carried out by removing the sucrose and chlorogenic acid components. Figure 3 shows the distribution of metabolites from coffee samples that have had the sucrose and chlorogenic acid components removed (extended treatment). It can be seen that the clustering analysis has been able to separate sample groups A with B and C. From this data, it can be seen that sample A has a different distribution of metabolites when compared to samples B and C. This finding shows that coffee grown in the lowlands will produce different metabolites. The dominant compounds in sample A are compounds with numbers 16, 19 and 37. These compounds are 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime, Scyllo-Inositol, 1-Monopalmitin and D-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxyme, (1Z)-. Coffee might contain a variety of chemical compounds that affect its flavor, aroma, and texture, including Scyllo-Inositol, 1-Monopalmitin, and D-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-O-methyloxyme, (1Z)-. Since the distribution of metabolites in samples B and C is not separated in Figure 3, further analysis was carried out to see the clustering of compounds in samples B and C. Do samples B and C really have the same metabolite distribution? Figure

5 shows a more focused distribution of metabolites between samples B and C. It can be seen that, if the comparison is only done on samples B and C, the distribution of metabolites can occur in more detail. Samples B and C can be separated due to the distribution of existing metabolites. It can be seen that 4 compounds seem to affect the clustering of sample B against C. The compounds have identities with no. 4, 9, 12 and 17 with the names Glycerol, Citric acid, D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime and Stearic acid. Citric acid is one of the organic acids naturally found in coffee beans, especially Arabica varieties, and contributes to the fresh, slightly citrusy acidity of coffee. D-fructose, or fructose, is one of the natural sugars in coffee that contributes to its sweetness, although much of it degrades during roasting and contributes to the formation of aroma compounds through the Maillard reaction. 1,3,4,5,6-pentakis-O-(trimethylsilyl)-O-methyloxime is a derivative compound that is often detected in the analysis of the chemical composition of coffee using chromatography techniques, indicating the presence of various sugars and volatile metabolites that affect the complex aroma of coffee. Meanwhile, stearic acid, or stearic acid, is a saturated fatty acid found in small amounts in coffee, contributing to the characteristics of oil in coffee and the stability of foam in espresso. The combination of these compounds plays an important role in creating the balance of flavors, aromas, and textures that make coffee a complex and interesting drink (Bastian *et al.*, 2021; Yeager *et al.*, 2021; Seninde *et al.*, 2020). The findings of this study are very interesting, because they provide information that the growing position of the coffee tree will affect the profile of the compounds formed. The position of the tree growing at the highest altitude produces different compounds from plants growing at low altitudes. These findings certainly need to be verified further.

***Conclusion*:** GCMS analysis of Robusta coffee grown at three locations indicated that altitude affects the components in the green coffee. The higher altitudes (B and C) produced compounds like Glycerol, Citric acid, D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime and Stearic acid, which can be marker compounds in relation to the metabolic processes. Furthermore, in coffee plants grown in the lowlands (A), the compounds like 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime, Scyllo-Inositol, 1-Monopalmitin and D-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxyme, (1Z)- were the dominant components. The relationship between metabolic processes and the emergence of the above compounds can be analyzed more deeply through a metabolomics approach. Of course, this analysis provides a comprehensive explanation regarding the relationship between the location of a plant's growth and the distribution of important compounds formed. Further, this study will assist researchers and farmers in selecting high-quality coffee beans for further development by identifying key metabolite profiles, enabling informed decisions on optimal bean sources.

***CRediT author statement*:** Fitria Indah Permatasari: wrote the manuscript, Novizar Nazir: field supervisor, Tuty Anggraini: farmer coordinator, James Hellyward: farmer coordinator, Chairat Techavuthiporn: research evaluation *and* Daimon Syukri: research supervisor.

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